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THE USE OF DRUGS IN RABBIT MEAT PRODUCTION. BENEFITS AND RISKS (Round Table)

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THE USE OF DRUGS IN RABBIT MEAT PRODUCTION. BENEFITS AND RISKS

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SUMMARY

Rabbits are one of the most important of the minor food animals. Veterinary drugs and feed additives are currently a critical component of rabbit meat production. Antimicrobial agents are used for three major purposes in rabbits : therapy (to treat an identified illness), prophylaxis (to prevent illness in advance) and performance enhancement (to increase feed conversion, growth rate or yield). For growth promoting purposes they are included in the feed at low levels and for therapeutic purposes they are used in feed usually at higher levels for their antibacterial, anthelmintic, antiprotozoal, antifungal effects. The minor species suffer the same kinds of diseases that those found among major species. However, to treat these infections only few drug products are labelled for rabbits. For producers, the only access to many drugs is through extra-label use of products developed for other species with the aid of the 'cascade' system. Other measures to find ways of resolving the drug availability for minor species included: (1) the creation of an European orphan drug fund in the veterinary sector financed by the Community budget to support provision of additional data to enable MRLs, and (2) the modification of Council Regulation 90/2377/EEC to grant protection to those companies which sponsored the residue evaluation of a substance, and limited in time.

Anatomy and physiology of digestive tract in the rabbit, in particular the coprophagy phenomenon, affect to drug pharmacokinetics specially absorption parameters, drug metabolic pathways as well as the pharmacokinetics of residues in edible tissues. Coprophagic habits may contribute to the observed prolonged period of absorption and the two-compartmental nature of the kinetic properties of some drugs after oral administration. Therefore, the establishment of the withdrawal time is an essential point to take in consideration in the rabbit.

The use of drugs and additives created both benefits and risks. With proper controls, the benefits should exceed the risks, and "new" risks will replace the "old" risks at a lower level of threat. The regulatory authorities have approved new antimicrobials for use in animals for therapeutic purposes as prescription-only products for several years. This prescription-only policy is based on authorities desire to assure the proper use of antimicrobials through precise diagnosis and correct treatment of disease to minimize animal suffering and to avoid unsafe residues. Antimicrobial products for use in animals must be stringent standards for safety, efficacy, and quality to be approved. In the past, microbiological safety studies which examined resistance patterns and pathogen load were only required for antimicrobials to be used in feed (e.g. prevalence of shed *Salmonella*). Due to concerns about antimicrobial resistance, the safety assessment now must include evaluation of resistance concerns with the conduct of pre-approval studies and post-approval monitoring programs. Residues of drugs or their metabolites in food products from treated food animals are major considerations in the safety of drugs approved for use in food animals. In Europe, regulatory authorities approve of drug dosages, routes of administration, durations of treatment, withdrawal times, and LMRs are designed to ensure the safety of foods derived from treated animals. EU regulations have effectively prevented allergenic, toxic, and potential toxic residues from entering the food supply.

INTRODUCTION

Veterinary drugs and feed additives are currently a critical component of rabbit meat production. They provide many benefits related to animal health, animal welfare, and economic return for the industry but the inappropriate use may pose an increased health risk to the people who consume the products from those animals. Since the benefits of subtherapeutic use of antibiotics in enhancing growth and feed efficiency in animals were first observed almost half a century ago, the number and use of these products has increased. In fact, the discovery of benefits of subtherapeutic use of antibiotics is often credited with the move toward more intensive animal production management systems to produce greater quantities of food but quality and safety of food production remain as two important aims. The health of food-producing animals is intrinsically linked to human health. That is to say, factors that affect food-animal health will, in turn, affect human health.

The use of drugs (in human as well as animals) and additives created both benefits and risks. With proper controls, the benefits should exceed the risks, and "new" risks will replace the "old" risks at a lower level of threat.

Public attention today focuses primarily on the favorable and unfavorable effects of animal drug use on human health. For livestoock producers and veterinarians, attention also is focused on the favorable and unfavorable effects of animal drugs use on animal health and on the consequences of the inadequate numbers of approved drug available for use. Antimicrobial substances are one class of drugs used extensively in food-animal production therapeutically and subtherapeutically. By fact the most important concerns among stakeholders today are microbial resistance to these compounds and residues of these compounds in the food supply.

ANATOMY AND PHYSIOLOGY OF DIGESTIVE TRACT. THE COPROPHAGY AS A PHENOMENON (PECULIARITY) OF THE RABBITS

The simple stomach of the rabbit which lacks specialised regions is thin-walled and large. The small intestine consists of the very long duodenum and the mesenterical small intestine or jejunum and ileum with the terminal portion of the latter expanded as the rounded sacculus rotundus. The ileum possesses a thinner wall and thus is more transparent than the more vascular jejunum and duodenum. The very large, thin-walled, coiled cecum terminates in the thick-walled light-colored vermiform process or cecum-appendix. The cecum is characterized by a spirally arranged constriction related to internal folding of the mucosa (spiral valve). The first part of the colon is replaced by or structured like the cecum and constitutes the ampulla caecalis coli. The colon is characterised by sacculation, or haustra, and the presence of taenia coli. The colon is continued through the pelvis as the rectum terminating in the anus. The average absolute lengths of the small intestine, cecum, and colon have been given as 3.56, 0.61, and 1.65 m, respectively. In the rabbit the intestine is 10 times longer than the body length itself (Weisbroth et al., 1974)

Rabbits normally excrete both hard and soft faeces; about 80% of the total excreted being of the hard type. Soft faeces are produced by the initial digestion of food. The soft faeces are normally consumed by the rabbit and excreted as the hard type. This physiological peculiarity of the lagomorpha is named coprophagy. By consuming the soft faeces, the rabbit apparently makes use of nutritive products resulting from metabolism of intestinal flora. Germ-free rabbits, however, do not consume their soft type faeces.

It is known that the biotransformation pathways of xenobiotic compounds, such as drugs or additives, are highly species-dependent. For this reason, coprophagy may have influence on xenobiotic behaviour throughout recycling of part of the caecal contents. The establishment of bioavailability and metabolic pathways of xenobiotis in the rabbit, and specially the pharmacokinetics of residues in edible tissues, for fixing a withdrawal time is an essential aspect to take in consideration. Also, in this respect, the studies should be performed with normal rabbit and with rabbits bearing collars designed to prevent coprophagy (i.e. noncoprophagous rabbits) for the comparison of the results.

A study was conducted with ^{14}C-robenidine to know the incidence of coprophagy on its bioavalilability. In this study, the kinetics of tissue residues showed that the total radioactivity leveled off after 6 days of continuous administration in most samples; during the depletion phase a fast elimination generally occurred but a longer persistance of the residues was notice in the liver. A comparison between normal rabbits and rabbits bearing collars led to the observation that coprophagy was involved in the bioavailability of robenidine and resulted in the recycling of 8.5% of the ingested substance (Tulliez et al., 1982). Pharmacokinetic and residue studies after oral administration of ^{14}C-salinomycin showed that salinomycin was rapidly absorbed from the rabbit's gut. Elimination occurred mainly via the faeces (56-80% within 3 to 8 days). In all, 8 to 15% was recovered in urine. These values were slightly influences by coprophagy (SCAN, 1999).

It should also be noted that coprophagic habits may contribute to the observed prolonged period of absorption and the two-compartmental nature of the kinetic properties of some drug distribution with oral administration. In addition, the low bioavailability of drugs in the rabbit may be attributed to the large food mass normally maintained in the stomach. For example, norfloxacin and enrofloxacin present a percentage of oral absorption lower in rabbits (Park et al., 1998; Broome et al., 1991) than in ruminant calves (Davidson et al., 1986) and poultry (Anadón et al., 1992,1995). In contrast, the results from rabbits and other species on the pharmacokinetic profile after intramuscular administration of drug products seem to be comparable. Ampicillin pharmacokinetic behaviour in rabbits after intramuscular dosing (Olling et al., 1995) was similar to that of the experiment in calves conducted by Nouws et al., (1982). On the basis of the results obtained with ampicillin, it has been postulated that the rabbit could be an animal model for estimating relative bioavailability and tissue tolerance of intramuscular drug products. However, to validate this rabbit model further investigations with other intramuscular drug products are necessary.

Rabbits are also used for teratogenicity studies and for some short term skin (i.e. acute skin) and eye studies (i.e. ocular irritation test, Draize). Several strains are widely available (New Zeland White, Dutch, etc.), with some now beginning to became available at SPF standards. However, the rabbit is not a good model to study the teratogenicity potential of antibacterials by its sensitivity to disturbance of intestinal microflora. Also, the rabbit is an animal model for measuring drug levels in the cornea, aqueous humor, iris, ciliary body, lens, vitreous humor and coroid-retina; rabbits have been used for testing drug distribution into lacrimal glands (Karcioglu, 1999).

RABBIT MEAT PRODUCTION AND DRUGS USE

Availability of medicines in rabbits (a minor species) : Rabbits are one of the most important of the minor food animals. The minor species suffer the same kinds of diseases that those found among major species. Rabbits, for example, have the same variety of

bacterial infections as do avian and mammalian species; the most frequently seen infections in rabbits are respiratory tract, gastrointestinal tract disease, and subcutaneous abscesses caused by fight wounds or other injuries. However, to treat these infections only a few drug products are labelled for rabbits (e.g. sulphonamides, enrofloxacin, kanamicin, colistin). Potential profit from such products usually is too small to recover the costs of developing them, often even when the active ingredient is already marketed for major species.

Undoubtedly, the lack of drugs, the discouraging marketing opportunities, and the limited research and information assistance from the regulatory authorities all contribute to contain growth of the minor-species industries. For producers, the only access to many drugs is through extra-label use of products developed for other species with the aid of the 'cascade' system (article 4.4 of Council Directive 81/851/EEC). The European authorities were requested to investigate possible measures to find solutions at a political and legal level. So, they proposed relaxation of this 'cascade' system by an amendment of the Directive so that any products authorised in one Member State for any species, with an MRL established for the active ingredient in that species can be used in any other species within the same class (i.e. mammalian, piscine, avian) without an MRL being required in the new species; this might only be allowed where no other medicinal product is legally authorised. Anywise, the 'cascade' system refers to the exceptional application in one animal or in a small number of animals only. The provision should not routinely be given as the solution to the availability of authorised medicines for the minor species, where large numbers of animal species may need treatment and where the stated minimum withdrawal period is not adequate or appropriate.

Other measures to find ways of resolving the drug availability for minor species included: (1) the creation of an European orphan drug fund in the veterinary sector financed by the Community budget to support provision of additional data to enable MRLs, and (2) the modification of Council Regulation 90/2377/EEC to grant protection to those companies which sponsored the residue evaluation of a substance, and limited in time. Any immediate action to salvage substances can only then be considered if the substances in question are used in treatments where no alternative is available.

The aim of Council Regulation 90/2377/EEC, namely is: (a) to protect consumers from potentially health-threatening residues of veterinary medicinal products in foodstuffs, (b) to promote uniform establishment of maximum limits for veterinary drug residues in foodstuffs, and (c) to facilitate intra-community trade in foodstuffs of animal origin and avoid potential distortions of competition between Member States of the European Union.

Under European Union legislation, all pharmacologically active substances used in food-producing animals must be entered into one of three annexes of Council Regulation 90/2377/EEC. These are: annex I, full MRLs; annex II, no MRLs required on consumer safety grounds; annex III, provisional MRLs with an established expiry date (pending further data not relating to major aspects of concern relating to safety). These outstanding issues are likely to relate to provision of fully validated analytical data. Annex IV, is the destination of drug considered unsafe on consumer health grounds. Drugs in this last annex are effectively prohibited for use in food-producing animals within the EU. Recently another conclusion may be that due to the insufficiency of the data provided, no recommendation for inclusion in any of the annexes can be made; the net result in the latter case is the same as the inclusion in annex IV.

The standard approach to assessing the safety of residues in foodstuffs intended for human

consumption is based on the determination of the acceptable daily intake (ADI) on which in turn MRLs are based. The basis for the calculation of the ADI is the no-observable-effect-level (NOEL) with respect to the most sensitive parameter in the most sensitive appropriate test species identified from a battery of toxicology studies, or in some cases, where such data are available, from observations in humans. A safety factor is then applied to provide a margin of safety, taking into account the inherent uncertainties in extrapolating animal toxicity data to human beings and to take account of variations within the human species. The ADI is an estimate of the residues, than can be ingested daily over a lifetime without a health risk to the consumer.

The MRLs should be established, according to the tissue distribution for the major edible tissues (muscle, fat, liver and kidney) where the concentrations would be sufficient, but in any case for one of the edible tissues of the carcass (muscle or fat) and one of the edible tissues of the offal (liver or kidney). An MRL is necessary for surveillance purposes (Anadón, 1990; Anadón and Martínez-Larrañaga, 1999).

The Regulation 90/2377/EEC entered in force on 1 January 1992. The original requirements was that the procedures to establish the limits for all existing substances were to be completed by 1 January 1997. That having proved impossible, Council Regulation 97/434/EC of 3 March 1997 amending Regulation 90/2377/CEE extended the deadline until 1 January 2000 for substances for which a valid application for the establishment of a MRL had been lodges. For certain substances, however, extension was granted only until 1 January 1998.

In general, the substances included into one of the three annexes (i.e. I, II and III) for 'all mammalian food species' can be used in rabbits such as ampicillin, cloxacillin, oxytetracycline, chlortetracycline, tetracycline, febantel, and levamisol.

For rabbits, some substances are no longer available as veterinary medicines, e.g. furazolidone and nitrofurans (except furazolidone) which were included in annex IV of Regulation 90/2377/EEC due to safety concerns. Nitrofuran derivatives are synthetic compounds that possess antimicrobial activity (sentitive: some Gram positive and Gram negative bacteria; insensitive: *Pseudomonas* spp. and some *Proteus* spp.) including nitrofurazone, nitrofurantoin furaltadone and furazolidone. Alternatives for nitrofuran derivatives in rabbits are the sulphonamides (e.g. sulphaquinoxaline) and those drugs available against infections of Gram positive and Gram negative bacteria. Chloramphenicol is also no longer available as veterinary drug because this drug constitute a hazard for the consumer health (Anadón, 1985). However, examples for alternatives are thiamphenicol and florfenicol but the companies did not defend the MRLs for rabbits because there was litter commercial return.

Thiopental sodium and thiamylal recently have been proposed to entry in annex II for general anaesthesia in rabbits. Other substances included in annex II for rabbits are alfaprostol (extension), apramicin and lecirelin. The criteria for inclusion of substances in annex II of Council Regulation 90/2377/EEC are the following: (1) the substance is of endogenous origin/is a normal component of the diet in humans/ is generally recognised as safe for humans, (b) the substance is used in a small number of individual animals, infrequent or non-regular treatments, (c) the animals are unlikely to be sent for slaughter during or immediately after treatment, (d) poor or absent absorption from the GI tract or from sites of local application, (e) the substance is rapidly and extensively detoxified or excreted.

Other substances such as nalidixic acid, pyrimethamine, fluanisone + fentanyl are not available as veterinary medicines, and where no MRL application has been made (non-defended substances). These substances were previously authorised as veterinary medicines in at least one EU Member State. Finally, substances such as olaquindox, itraconazole, and ipronidazole were never authorised as veterinary medicines in the EU for rabbits.

The extension to rabbits means that an applications has been submitted for the extension of the MRLs, according to a general strategy, based on the MRLs in major species (EMEA, 1997).

Tables 1 and 2 present substances included in annexes I and III exclusively for rabbits.

Table 1.- Annex I of the Council Regulation 90/2377/EEC

Pharmacologically Active substances(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Enrofloxacin	Sum enrofloxacin and ciprofloxacin	Rabbit	100 mg/kg 100 mg/kg 200 mg/kg 300 mg/kg	Muscle Fat Liver Kidney	-

Table 2.- Annex III of the Council Regulation 90/2377/EEC

Pharmacologically Active substances(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Kanamycin	Kanamycin	Rabbit	100 mg/kg 100 mg/kg 600 mg/kg 2500 mg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.1.2000
Colistin	Colistin	Rabbit	150 mg/kg 200 mg/kg 150 mg/kg 150 mg/kg	Liver Fat Muscle Fat	Provisional MRLs expire on 1.7.2000
Aminosidine	Aminosidine	Rabbit	500 mg/kg 1500 mg/kg 1500 mg/kg	Muscle Liver Kidney	Provisional MRLs expire on 1.7.2000

In EU, implementation of 90/2377/EEC means that as from 1 January 2000 the only pharmacologically active substances that may be used in veterinary medicinal products for food-producing animals are in principle those for which MRLs have been established in accordance with Regulation 90/2377/EEC and included in annex 1, II or III to the Regulation. Many substances used in rabbit medicine are already non-available. It is now a reality that certain of these drugs withdrawn from the market will affect clinical indications for which no veterinary medicinal product is legally available leading to illegal use of other medicines with likely consequences of concern for human consumer. Therefore, the ‘therapeutic crisis’ remains most acute for minor species; in some Member States what may be a minor species in one country may be a major species in another e.g. rabbits, sheep and goats in southern European countries. Nevertheless, we need to continue to ensure the same high standard of consumer protection in the future.

An update of the position paper on availability of veterinary medicines presenting recommendations in respect of the problem that insufficient medicinal products are available for the treatment of certain diseases and certain animal species have been recently released and it can be consulted by internet (EMEA/CVMP/130/00).

BENEFITS AND RISK TO ANIMALS

Benefits of antimicrobial use: In the last years, the use of antimicrobials in feedingstuffs has increased in line with the development in intensive animal production. The word antimicrobial is often used to encompass any substance of natural, semi-synthetic or synthetic origin that kill or inhibits the growth of a microorganism. With respect to spectrum of activity, antimicrobial substances may be divided into antibacterial, anthelmintic/antiprotozoal, antifungal and antiviral drugs. Antimicrobial agents are used for three major purposes in animals : therapy (to treat an identified illness), prophylaxis (to prevent illness in advance) and performance enhancement (to increase feed conversion, growth rate or yield). For growth promoting purposes they are included in the feed at low levels and for therapeutic purposes they are used in feed usually at higher levels for their antibacterial, anthelmintic, antiprotozoal, antifungal effects.

Therapy usually involves and individual animal or a defined group of diseased animals while prophylactic treatment involves the treatment of a group of animals. The purpose of the latter is to prevent diseases that might otherwise occur. A special form of prevention, also called for some people metaphylaxis, is when all animals in a group of animals are medicated, in situations when the proportion of animals diseased during a defined time period reaches a threshold value. In such situation, the probability of most, or all, of the animals getting infected is high. In both therapy and prophylaxis, the drug is administered over a defined, preferably short, period of time and in both instances the drug is used upon prescription. The dosages used must be high enough so that concentrations that are inhibitory for the infectious agent are reached at the site of infection.

In the European Union, coccidiostats are incorporated in the feed, under the category of ‘Coccidiostats and other medicinal substances’. Coccidiosis is the most serious parasitic disease of colonized rabbits; bile duct epithelium is parasitized by *Eimeria stiedae* and intestinal epithelium is parasitized by several species of *Eimeria*, some of which are pathogenic and some nonpathogenic. Among the coccidiostat feed additives used in rabbits are the meticlorpindol (125-200 ppm of complete feeding stuffs), meticlorpindol + methylbenzoquate (220-220 ppm of complete feeding stuffs), robenidine (50-66 ppm of complete feeding stuffs), and salinomycin (15-25 ppm of complete feeding stuffs). For salinomycin no withdrawal period is fixed, however for the others coccidiostats 5 days of withdrawal period are established.

A fundamental hypothesis of pharmacokinetics is that a relationship exists between the pharmacological or toxic response to a drug and the accessible concentration of the drug (e.g. in blood). This hypothesis has been documented for many drugs, although it is apparent for some drugs that no clear or simple relationship has been found between pharmacological effect and concentration in plasma. In most cases, the concentration of drug in the systemic circulation will be related to the concentration of drug at its sites of action. The pharmacological effect that results may be the clinical effect desired, a toxic effect, or in some cases, an effect related to efficacy or toxicity. Clinical pharmacokinetics attempt to provide both a more quantitative relationship between dose and effect and the framework with which to interpret measurements of concentrations of drugs in biological

fluids (Anadón et al., 1999).

The three most important pharmacokinetics parameters are: clearance, a measure of the body's ability to eliminate drug; volume of distribution, a measure of the apparent space in the body available to contain the drug; and bioavailability, the fraction of drug absorbed as such into the systemic circulation. Of lesser importance are the rates of availability and distribution of the agent (Benet et al., 1996). When choosing an effective antimicrobial regimen information regarding organism-specific parameters such as minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) need to be considered in conjunction with specific pharmacokinetic parameters.

Low levels of antibiotics for growth promotion produce an improvement in general health status. Improvements in average daily gain (+ 3,39%) and in feed conversion rate (- 5.03%) were observed using the antimicrobial feed additive flavophospholipol (flavomycin) in growing rabbits (approved in the EU at a range between 2-4 ppm of complete feeding stuffs) (Rebolini et al., 1982). Apart from reducing the time for animals to reach market weight, thus lowering overhead costs on buildings and labour, the effects of saving feed can be highly significant. There are three mechanisms of action through which antibiotics appear to enhance growth and production (Cromwell, 1991). The first involves direct biochemical events that are affected by antibiotics: nitrogen excretion, efficiency of phosphorylation reactions in cells, and direct effects on protein synthesis. The second involves direct effect on metabolism, including the effects of antibiotics on the generation of essential vitamins and cofactors by intestinal microbes and the way that antibiotics affect the populations of microbes that make these nutrients. In addition, feeding of antibiotics is associated with a decrease in gut mass, increased intestinal absorption of nutrients, and energy sparing. The third proposed mechanism of action is eliminating subclinical populations of pathogenic microorganisms.

The public has long-standing concerns over potentially harmful drug residues in foods. The possibility that chemical additives, drugs and their metabolites (drug residues) could cause allergic reactions or diasease is not taken lightly by the public or by health care professional.

Toxicity of antimicrobial drugs: The use of antimicrobial drugs in rabbits is associated with a high incidence of undesirable side-effects. Clindamycin, lincomycin, erythromycin, and narrow-spectrum penicillins can produce fatal adverse reactions through an indirect effect on gastrointestinal micro-organisms. Lincomycin administered either orally or parenterally is known to be highly toxic to rabbits (Gray and Lewis, 1966). Experimentally, oral administration of lincomycin at a dosage of 30 mg/kg bw per day and of clindamycin (a lincosamide derivative of lincomycin) at a dosage of 15 mg/kg bw per day induced diarrhea and death in 12/12 and 25/25 rabbits (Katz, et al., 1978). Severe diarrhea has been induced in rabbits with oral dosages of lincomycin as low as 5 mg per animal per day. Following a single IV injection of 0.5 mg lincomycin/kg bw, 5 out of 10 rabbits either died or were killed for humane reasons within two weeks of dosing, and 7 out of 10 rabbits had died by one and a half months. In oral toxicity studies, groups of rabbits (3 per group) were administered lincomycin HCl. Only the lowest dose of 0.5 mg/kg bw was without mortality. All other treatment groups (5, 50, 100, and 150 mg/kg bw) resulted in mortalities, such that by four weeks, 9 out of 15, and 12 out of 15 rabbits had died. Pathology revealed gastrointestinal stasis and, for those animals that died, hemorrhagic suffusion of the serosal surface of the cecum (Gray et al.1964). The observed toxicity was interpreted as resulting from a (Gram-positive) gastrointestinal flora imbalance following the lincomycin administration

Penicillin and erythromycin have also been reported to cause diarrhea and death in rabbits (Gray and Lewis, 1966). Ampicillin administered for 6 days in the drinking water at a mean daily dosage of 10 mg/kg bw, killed 8 of 16 rabbits so treated (Thilsted et al., 1981).

There are various possibilities for ingestion of lincosamide or penicilllin antibiotics by rabbits, like mix-ups or contamination of feed at the feed mill, inadvertent feeding or inclusion of poultry litter in animal feed (e.g. amoxycillin and lincomycin-spectinomycin are the antibiotics most commonly prescribed for the treatment and prevention of necrotic enteritis in poultry). The susceptibility to accidental intoxication varies between different non-target species. For example, rabbits and horses are generally very sensitive to disturbances of intestinal microflora by antimicrobial substances. Such disturbances may, in severe cases, cause fatalities.

Polyether ionophore antibiotic group including monensin, narasin and maduramicin are considered as toxic drugs for rabbits. The contamination of feed processing lines by previous poultry medicated feed is the origin of rabbit feed pollution. In these cases intoxications have also been observed in rabbits, which showed a nervous syndrome and severe hepatic degeneration adjudged to narasin and maduramicin respectively.

It is well accepted that the mechanism by which lincomycin and penicillin induce diarrhea in rabbits is through alteration of the bacteria flora of the cecum. The antibiotics selectively destroy Gram-positive aerobes and certain Gram-negative anaerobes, and this action allows marked proliferations of nonsensitive bacteria, particularly coliforms and certain species of clostridia. An enterotoxin produced by *Escherichia coli* or *Clostridium* sp is thought to be directly responsible for the signs and lesions (Gray et al., 1964; Gray and Lewis, 1966).

BENEFITS AND RISK TO HUMAN HEALTH

Benefits of antibiotic use: The antibiotics are used in food-animal production for the primary benefit of (1) the health and welfare of the animal, (2) carcass quality and overall efficiency of growth and production, (3) economics, and (4) human public health. The benefit to human health in the proper use of antibiotics in food animals is related to the ability of these drugs to combat infectious bacteria that can be transferred to human through direct contact with the sick animal, through consumption of food contaminated with pathogens, or through proliferation in the environment. The advantages of antibiotic use in animals are related to the prevention of overt bacterial disease and improvement in animal performance through reducing the physiological costs of limiting growth that are incurred in the process of fighting low-level and overt disease. Those limitations need to be minimized to permit better nutrient use, enhanced growth rate, and feed efficiency. However, because of the controversy surrounding the development of antibiotic drug resistance in animal and human populations, and because of the consequences for human health and clinical practices, use of antibiotic drugs in food-producing animals has been questioned by the FDA, policy makers, health care professionals, and consumer organizations, among others, and has been studied regularly since 1960s as directed by several agencies. Some groups have argued for a substantial reduction in the use of antibiotic drugs in food-animal production. Other contend that microbial contamination of animal-food products would increase without the use of these drugs.

Possible hazards of antibiotic use: The use of antimicrobials is clearly a determining factor in the emergence of resistance. Since resistance against antimicrobial feed additives could transfer between different bacteria and between human and animal hosts, there is a

concern that the use of antimicrobial feed additives could contribute to the increase of the environmental pool of resistance genes. The resistance among enterocicci to glycopeptide antibiotics has attracted particular concern. High level resistance to glycopeptides mediated by the *van*A-gene cluster has been detected in *Enterococcus faecium* and other enterococcal species. Particular importance attaches to the emergence of the high-level and cross-resistance to vancomycin (used in human therapy against infections caused by enterococci) and teicoplanin. Glycopeptides are looked upon as important reserve antibiotics for treatment of infections caused by ampicillin-resistant enterococci and by staphylococci with multiple antibiotic resistance. Reports from various European State members have documented outbreaks of infections by vancomycin-resistant enterococci (VRE) occurring both within and by transfer between hospitals (Anadón and Martínez-Larrañaga, 1999). According to some scientific papers, the use of avoparcin leads to the selection of resistant strains in the animal and this form of antibiotic resistance can present problems for antibiotics used in human therapy. For this reason, the EU suspended the use of avoparcin in April 1997 (precautionary principle)(Directive 97/6/EC). Subsequently, was extended the ban to include the use of the macrolides, tylosin and spiramycin, the streptogramin, virginiamycin and the polypeptide, bacitracin-zinc (Regulation 98/2821/EC) because was claimed that tilosin results in development of erythromycin cross-resistance since clinically most common resistance mechanisms within these macrolides and streptogramins are similar (Anadón and Martínez-Larrañaga, 1999). It should indicated, substances such as macrolides and streptogramins were never authorised as antimicrobial feed additives in the EU for rabbits.

The EU precautionary principle used to suspend the antimicrobial feed additives should be considered within a structured approach to the analysis of risk which comprises three elements: risk assessment, risk management, risk communications. The precautionary principle is particularly relevant to the management of risk. This principle, which is essentially used by decision-makers in the management of risk, should not be confused with the element of caution that scientist apply in their assessment of scientific data. Recourse to the precautionary principle presupposes that potentially dangerous effect deriving from a phenomenon, product or process have been identified, and the scientific evaluation does not allow the risk to be determined with sufficient certainty (European Commission, 2000).

In general, the use of antimicrobial drugs in animals selects for resistant bacteria. These resistant bacteria, if transferred to people via food or the environment, can have an adverse effect on human health. This effect can be direct, if the resistant bacteria are themselves human pathogens, or indirect, if the resistant bacteria are not human pathogens but instead transfer their resistance genes to human pathogens. Antimicrobial resistance sometimes develops in enteric bacteria that contaminate food and cause human illness (D'Aoust, 1997; Nachamkin, 1997). When food borne infections are caused by a resistant pathogen, medical treatment may be compromised. For example, the use of fluoroquinolones to treat various respiratory diseases in poultry has lead to the development of fluoroquinole-resistant *Campylobacter* in the intestinal tract of birds treated. In the rabbit, *Campylobacter*-like organisms have been described in young rabbits with acute typhlitis (Fraser et al., 1991). In poultry, *Campylobacter* from the intestinal tract can contaminate the carcass at slaughter and during processing. Improperly cooked poultry and improperly handling poultry are vehicles for *Campylobacter* infections in humans.

Antimicrobial resistance sometimes develops in enteric bacteria that contaminate foods but does not typically cause human illness (Bates et al., 1994). When humans ingest resistant enteric bacteria of food animal origin, the resistance genes can be transferred to bacteria

indigenous to the intestinal tract of humans. Bacteria indigenous to the human intestinal tract frequently cause human disease. If these indigenous human bacteria become resistant to drugs used in human therapy, human health may be compromised due to limited therapeutic options. *Salmonella* apparently can develop resistance quickly when fluoroquinolones are used in both human and veterinary medicine. In addition, bacteria present in the intestinal tract of the rabbit at slaughter, including *Salmonella* and *Eschericha coli*, can contaminate food and cause human illness. Generally, antimicrobial drug therapy cures clinical infections as mentioned previously by reducing the level of specific pathogens. However, this therapy may also disturb the normal intestinal microbial ecosystem in the animal causing an increase in the bacteria that cause human infections or duration of the carrier state of such bacteria (pathogen load), thereby increasing the potential for contamination of food and consequent human illness (Aserkoff and Bennett, 1969). In conclusion, based on the above, to assess this potential human health impact of the microbial effects associated with all uses of all classes of antimicrobial new animal drugs intended for use in food-producing animals, it may be necessary to evaluate the following two separate, but related aspects (1) the rate and extent of development of antimicrobial drug resistant enteric bacteria formed in the animal's intestinal tract following exposure to the antimicrobial new animal drug (resistance), and (2) changes in the number of enteric bacteria in the animal's intestinal tract that cause human illness (pathogen load).

The regulatory authorities have approved new antimicrobials for use in animals for therapeutic purposes as prescription-only products for several years. This prescription-only policy is based on authorities desire to assure the proper use of antimicrobials through precise diagnosis and correct treatment of disease to minimize animal suffering and to avoid unsafe residues. Antimicrobial products for use in animals must be stringent standards for safety, efficacy, and quality to be approved. In the past, microbiological safety studies which examined resistance patterns and pathogen load were only required for antimicrobials to be used in feed (e.g. prevalence of shed *Salmonella*). Due to concerns about antimicrobial resistance, the safety assessment now must include evaluation of resistance concerns with the conduct of pre-approval studies and post-approval monitoring programs. It is critical that prudent use of antimicrobials be emphasized to minimize the development of antimicrobial resistance and to ensure the continued efficacy and availability of antimicrobial products for use in food-producing animals. For this reason, it has been defined prudent use of therapeutic antimicrobial agents as the use that maximizes therapeutic effect while minimizing the development of resistance (Anadón et al., 1999a).

The prudent use and the limitation in the volume of drugs used, therefore seem to be a rational response to limit the problem. The objective is to optimize therapeutic efficacy and minimize resistance to antimicrobials to protect animal and public health in the long-term. The mechanisms employed involve a combination of alternative preventive strategies (e.g. improved husbandry, hygiene or immunisation), use of narrow spectrum antibiotics, improved diagnosis leading to better choice of therapeutic agent, minimizing environmental contamination and duration of therapy and increasing control over the availability of antimicrobials.

Limiting the prophylactic use of antibiotics is an importante means of decreasing total usage.

Of major epidemiological significance in reducing the risks of transfer of resistant zoonotic strains are the points of possible cross-contamination, these include the abattoir, meat preparation surfaces (both retail and in the home), inadequate cooking and persons in

regular contact with animals carrying these infections. Identifying and removing sources of infection and strict hygiene precautions are the surest means of reducing disease incidence.

Possible alternatives to antibiotics: Producers would find ways of producing rabbits without the use of antimicrobial agents. One possibility that has been suggested as an alternative to antibiotics is to switch to "promicrobial agents", commonly called "probiotics". These are live (viable), naturally-occurring microorganisms that are fed directly to animals, and are now more appropriate called "direct-fed microbials". They include a number of microbials, but the more common ones are species of *Lactobacilli* and *Enterococci* (formely called Streptococci), and yeast (*Saccharomyces cerevisiae*). They are throught to beneficially affect the host animal by modifying the microflora in the gut to a more desirable balance. In the EU, *Bacillus cereus* var. *toyoi* (preparation of *Bacillus toyoi* var. *toyoi* having a minimum activity of 1 x 10^{10} UFC) is used in rabbits for fattening and for reproduction at levels in 0.1 x 10^9 to 5 x 10^9 UFC per kg of complete feeding stuffs and *Sacharomyces cerevisae* (preparation of *Sacharomyces cerevisae* having a minimum activity of 5 x 10^9 UFC) is used in rabbits for fattening at levels in 2,5 x 10^9 to 5 x 10^9 UFC per kg of complete feeding stuffs.

Another group of compounds, sometimes referred as prebiotics that may have beneficial effects are the oligosaccharides. The mannan-oligosaccharides (MOS) are small polymers of mannose that are found in relatively high concentrations in yeast cell walls. These compounds have a high affinity for specific sites in the intestinal wall that also bind pathogenic microbes; therefore they compete for the binding sites in the gut and prevent pathogens from colonizing the tract. This process is commonly referred to as 'competitive exclusion'. Fructooligosaccharides (FOS), short-chained polymers of fructose, have also recently been found to alter the gut microflora and stimulate growth. FOS differs from MOS in that it serves as an excellent substrate for the more desirable gut microflora such as the *Bifidobacteria*, and their rapid growth inhibits colonization on gut walls and prevents colonization of pathogenic microbes.

HUMAN HEALTH RISK FROM DRUG RESIDUES IN FOODS

The toxicity of drugs is an inherent part of all uses of medication, and there are differences from one animal or human to another, especially in allergic reactions. Residues of drugs or their metabolites in food products from treated food animals are major considerations in the safety of drugs approved for use in food animals. In Europe, EMEA or Member States approve of drug dosages, routes of administration, durations of treatment, withdrawal times, and LMRs are designed to ensure the safety of foods derived from treated animals. EU regulations have effectively prevented allergenic, toxic, and potential carcinogenic drug residues from entering the food supply.

Any adverse reaction is likely to occur due to acute rather than long-term chronic effects. The acute impact of antibiotics is not directly examined in the toxicological studies required in the pre-approval process for veterinary drugs, since the primary concern has focused on chronic effects (i.e. carcinogenesis).

Acute effects of foodborne drug residues on human health has also been described. These acute effects are defined as those that occur or develop rapidly after single administration of chemical substances. Some examples are the hipersensitivity and teratogenic effect. Allergic reactions to the beta-lactam drug penicillin, aminoglycosides, sulphonamides, and tetracyclines have been described in sensitive persons. Reportedly, both epidemiologic and experimental data indicate that levels of penicillin as low as 5 to 10 IU are sufficient to

produce an allergic reaction in previously sensitized individuals (Sundlof, 1994). Adverse human reactions are manifested as severe swelling of the skin, serum sickness, shock and less serious reactions such as skin rashes, asthma, and fever. Since these reactions occur at very low doses, it is highly probable that allergic individuals to the drug previously enumerated, when exposed via the food could suffer an allergic reaction. Probably the reason that few cases are documented is that many cases might be masked with other health conditions, specially in elderly populations, as well as problems with under estimation and under reporting.

Another acute toxicity effect that may occur as a result of exposure to violative animal drug residues is a teratogenic effect. Teratogens are active at very low doses and even brief exposure during a critical period of development can result in a deformation which lasts a lifetime. The changes of a woman at the critical stage of pregnancy coming in contact with a violative residue are very low.

Effects on human intestinal microflora: The standard human food safety assessment for new animal drugs accurately determines the safe concentration for traditional toxicological endpoints as mentioned above. However, the impact of low levels of antibiotics on the intestinal microflora is not directly examined in these toxicology studies. Therapeutic doses of antibiotics can cause adverse effects on the intestinal microfloral ecology (i.e. disruption of the intestinal microflora, or effects on the metabolic activity of intestinal microflora). The adverse effects of antimicrobials are a concern because of the important role that the intestinal microflora plays in maintaining and individual's health. Also perturbation of the intestinal microflora may compromise the effectiveness of other drug therapies and thereby adversely affect public health. Most studies of antimicrobial drugs and their effects on the human intestinal microflora were performed with therapeutic levels of antibiotics. In contrast to the well-documented negative effects of therapeutic dose of antibiotics, the effect of low levels (i.e. ppb or ppm) of antibiotics on perturbing the intestinal microflora is not well defined. It is possible that low doses of antimicrobials agents, such as those found as residues in foods, could alter intestinal enzyme activity and have an effect on certain hormones and drugs, since in most cases the lowest doses at which the perturbations in the intestinal microflora occur have not been determined. In order to ensure human food safety, FDA's CVM considered data gathered from a large number of compounds and determined that the maximum safe concentration for antimicrobial products is 1 ppm in a total diet of 1.5 kg. This equals a maximum antibiotic dose of 1.5 mg/d from consuming residues in food. This level of an antimicrobial residue in food should produce no effects on the intestinal microflora (Paige et al., 1997).

DRUG RESIDUES CONTROL IN ANIMAL PRODUCTS

Residue analysis within the framework programs are necessary to control not only the food stuffs 'meat' but also the living animal, both while it is being fattened and shortly before it is slaughtered. Urine and faeces can be used for analysis in the living animal. Samples of the feedstuffs must also be tested, as the manufacturers of animal feed stuffs must be integrated into corresponding control concept (Anadón and Martínez-Larrañaga, 1999). To ensure that MRLs established in the European Union are not exceeded, Member States carry out the control of residues in accordance with Council Directive 96/23/EC. According to Directive 96/23/EC Member States have to set up an annual plan for the control of residues in live animals and animal products. These plans lay down in detail the substances and tissues (annex II of this Directive for rabbit meat) to be analysed, the analytical methods to be used and their detection limits, the level of actions and the number of samples for each substance, species and matrix. These plans are subsequently submitted

to the European Commission, for approval of their conformity with Directive 96/23/EC.

In principle, sampling of animal tissues should be relatively straighforward because the various organs or parts should be easily defined. The problems of fat tissue are common to all sampling recommendations, in particular in the rabbit where fat is not well defined. The Codex Committee on Residues of Veterinary Drugs in Foods in 1993 defined commodities of low fat products to calculate residue levels and MRLs derived, which include "Group 30 mammalian meats : where adhering fat is insufficient to provide a suitable sample, whole commodity, i.e. muscular tissues including adhering fatty tissues without bone, is analysed and the MRL applies to the whole commodity (e.g. rabbit meat)".

The national reference laboratories are in charge of the accomplishment of the plans and are responsible for all laboratories involved in the analytical work to follow international recognised quality assurance systems. The yearly results of the analytical residue monitoring are compiled by each Member State and submitted to the Commission by March the following year. Where analytical results indicate that residue concentrations have exceeded the MRLs, the Member State(s) concerned shall, without delay, obtain all information on the animal and farm of origin and all details of the examination and results. If the controls carried out would demonstrate the need for an investigation or action in one or more Member State(s), the European Commission shall be informed immediately.

When submitting an application for the establishment of MRLs, the applicants are requested to submit a validated analytical method, which can be used for official residue monitoring and surveillance ('regulatory method').

Article 15 of the Directive 96/23/EC requires that whenever an authorisation is issued for the placing on the market of a veterinary medicinal product intended for the administration to a food production animal species, the competent authorities shall forward the routine analytical methods as laid down in Directive 81/851/EEC and Regulation 90/2377/EEC to the Community and national reference laboratories for detection of residues. The Member States to implement national plans for the control of residues in food-producing animals and their products use three categories of analytical methods such as: screening, confirmatory, and reference methods.

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